



Instructions for use

ROTI®Mark TRICOLOR XTRA

ready-to-use

Tricolor prestained protein molecular weight marker for SDS-PAGE

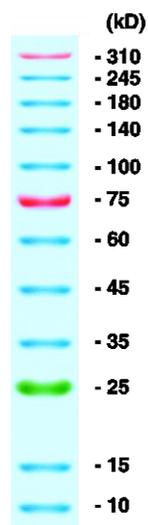


Figure:
ROTI®Mark TRICOLOR XTRA in Tris-Glycine gel, unstained. Resolution and distinctness of bands may vary depending on the gel and buffer system used.

I. Introduction

ROTI®Mark TRICOLOR XTRA is composed of nine proteins which are covalently coupled with a blue dye. In order to optimise orientation in the gel as well as control of running and transfer behavior three reference proteins were added, one protein prestained in green and with an apparent MW of 25 kD and two proteins prestained in red with an apparent MW of 75 kD and 310 kD, respectively.

ROTI®Mark TRICOLOR XTRA need not be stained in gel and is, therefore, ideal for checking transfer efficiency during Western-Blot and the gel run during electrophoresis.

The concentration of the individual proteins is set in such a way that homogeneous band intensity and optimal band sharpness can be obtained in unstained gel.

The proteins are denatured in reducing Laemmli buffer with 200 mg/ml (3.6 M) urea.

The molecular weights of the proteins change as a result of being coupled with the dye and vary according to the coupling efficiency. Additionally, the behaviour of the prestained proteins in the gel differs slightly depending on the actual gel and buffer system used. The indicated molecular weight values are, therefore, only approximate. The effective molecular weights may deviate from the values indicated.

Please note:

ROTI®Mark TRICOLOR XTRA, therefore, is not suitable for size determination of proteins in the gel.

II. Application

ROTI®Mark TRICOLOR XTRA is used for monitoring protein separation during SDS polyacrylamide gel electrophoresis and for controlling transfer efficiency of Western blot.

III. Composition

12 prestained proteins (0.1 – 0.4 mg/ml per protein) in aqueous Tris-Glycine buffer solution, modified according to Laemmli.

IV. Storage

- **The marker should be stored for a longer period at temperatures below freezing point!**
- **The marker will not be shipped cooled or with dry ice. This does not affect usability.**
- ROTI®Mark TRICOLOR XTRA can be stored at +4 °C for up to three months. Nevertheless, we recommend a temperature of -20 °C for long-term storage. To avoid frequent freezing and thawing, aliquots should be frozen.
- Normally, it is not necessary to heat the marker before use. Aggregate bodies may form when used for a longer period or when stored at +4 °C. These can be dissolved if heated for a short period (5 min, 80 °C).

V. Gel loading

- Recommended loading amount for mini gels (10 %; 0.75 mm thick): 3 - 5 µl
Western transfer: 1.5 – 2.5 µl
Silver staining: approx. 1 µl
Bands visible in volumes as low as 3 µl per lane.
- **Important:** Loading amount required varies depending on gel thickness, C/T ratio and width of comb tooth.

IV. Trouble Shooting

Marker bands cannot/can only be seen very weakly.

- Please ensure the correct loading amount. The recommended quantity is valid for mini gels with a thickness of 0.75 mm. If thicker or larger gels are used, the loading amount must be increased.
- Few weak marker bands: Under certain conditions marker proteins may agglutinate. Resolubilise marker aliquots by incubating for 5 min at 80 °C (alt. 1 min at 95 °C). Mix carefully.

Marker bands are fuzzy.

- Avoid overloading the gel!
- Please ensure that the marker is not stored at room temperature for a longer period. Place the marker on ice between two gel runs.
- Avoid frequent freezing/thawing of marker.
- Long-term storage should always take place at -20 °C.
- Please take care that the gel contains no air bubbles when casting.
- When casting the gel, please ensure that the gel solution is mixed thoroughly.
- Only use high quality acrylamide solutions, e.g. ROTIPHORESE® Gel 30 (Art. No. 3029) or Gel 40 (Art. No. 3030).
- Avoid overheating the gel. Reduce voltage if required.
- Check the composition and pH-value of the buffer used.

The marker is delivered in gel loading buffer and is immediately ready for use.

No heating, dilution or addition of reducing agents necessary!

Carl Roth GmbH + Co. KG

Schoemperlenstraße 3-5

76185 Karlsruhe

Postfach 100121

76231 Karlsruhe

Telefon: +49 (0) 721/ 5606-0

Telefax: +49 (0) 721/ 5606-149

E-Mail: info@carlroth.de

Internet: www.carlroth.de

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ROTI® Mark TRICOLOR XTRA

2244.1

250 µl

2244.2

500 µl (2 x 250 µl)